

NEW HOST RECORD, NEW RANGE INFORMATION, AND A NEW
PATTERN OF VOLTINISM: POSSIBLE HOST RACES WITHIN THE
HOLLY LEAFMINER *PHYTOMYZA GLABRICOLA* KULP
(DIPTERA: AGROMYZIDAE)

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Abstract.—The agromyzid leafminer *Phytomyza glabricola* is reported from the holly *Ilex coriacea*, a plant not previously reported to host leafminers. The known geographic range of *P. glabricola* is extended to include Alabama, Florida, Mississippi, North Carolina, and South Carolina. In these areas, it can be found feeding on sympatric populations of its hosts *I. coriacea* and *I. glabra*. *Phytomyza glabricola* reared from *I. coriacea* are univoltine, having only a single generation a year, while on *I. glabra* this species is multivoltine with at least two generations a year. This suggests that either this leafminer exhibits a high degree of host-associated phenotypic plasticity in life history or that host races are present.

Key Words: host race formation, sympatry, Aquifoliaceae, gallberry, inkberry

The agromyzid fly *Phytomyza glabricola* Kulp belongs to a complex of closely related leafminers, all of which feed on various species of holly in the genus *Ilex* (Aquifoliaceae) (Kulp 1968, Scheffer and Wiegmann 2000). *Phytomyza glabricola* was first described from the plant species *Ilex glabra* (Linnaeus) Gray by Kulp (1968) in his revision of holly-feeding *Phytomyza* species. On this host, *P. glabricola* makes a linear-blotch mine and is multivoltine (Kulp 1968, Al-Siyabi and Shetlar 1998). In 1996, I reared *P. glabricola* from 26 leafmines found on two bushes of *I. coriacea* (Pursh) Chapman growing in North Carolina. This plant species has not been previously reported to host agromyzid leafminers (Spencer and Steyskal 1986, Spencer 1990).

Ilex glabra and *I. coriacea* are shrubby evergreen hollies that grow sympatrically in

the coastal plain of the southeastern U.S. The range of *I. glabra* extends from coastal Nova Scotia to mid Florida to eastern Texas and Missouri and is slightly broader than that of *I. coriacea* which extends only from coastal Virginia to northern Florida to Texas [Galle (1997) includes Mexico in the range, but most other sources do not (Standley 1923, Lundell 1961, Gleason and Cronquist 1963, Radford et al. 1968, Correll and Johnston 1970)]. These two plant species are believed to be very closely related and go by the similar common names gallberry (*I. glabra*) and large gallberry (*I. coriacea*) (Galle 1997).

Phytomyza glabricola has been reported from Connecticut, Massachusetts, New Jersey, New York, Ohio, and Washington, D.C. (Kulp 1968, Spencer and Steyskal 1986), but not from the southeastern U.S. where both *I. glabra* and *I. coriacea* are

Table 1. Collection locations, hosts, and dates for *P. glabricola*.

Location	Host	Date
Alabama		
Covington Co.	<i>I. glabra</i>	Jan. 1998
Covington Co.	<i>I. coriacea</i>	Jan. 1998
Florida		
Calhoun Co.	<i>I. glabra</i>	Oct. 1999
Highlands Co.	<i>I. glabra</i>	Jan. 1998, Oct. 1999
Marion Co.	<i>I. glabra</i>	Jan. 1999
Wakulla Co.	<i>I. glabra</i>	Jan. 1998, Oct. 1999
Wakulla Co.	<i>I. coriacea</i>	Jan. 1998
Mississippi		
Forrest Co.	<i>I. glabra</i>	Jan. 1998
Forrest Co.	<i>I. coriacea</i>	Jan. 1998
North Carolina		
Brunswick Co.	<i>I. glabra</i>	Feb. 1997
Brunswick Co.	<i>I. coriacea</i>	Feb. 1997
Craven Co.	<i>I. glabra</i>	Feb. 2000
Craven Co.	<i>I. coriacea</i>	Feb. 2000
Moore Co.	<i>I. glabra</i>	Feb. 1996
Moore Co.	<i>I. coriacea</i>	Feb. 1996
New Hanover Co.	<i>I. glabra</i>	Feb. 1997, Jan. 1998
New Hanover Co.	<i>I. coriacea</i>	Feb. 1996, Feb. 1997, Jan. 1998
New York		
Suffolk Co.	<i>I. glabra</i>	Aug. 1996
South Carolina		
Berkeley Co.	<i>I. glabra</i>	Feb. 1997, Jan. 1998
Berkeley Co.	<i>I. coriacea</i>	Feb. 1997, Jan. 1998

common in the pine woods and bays of the coastal plain. The purpose of this study was to investigate the geographic range of *P. glabricola* and to determine whether this species commonly feeds on *I. coriacea*.

MATERIALS AND METHODS

From 1996 to 2000, I periodically traveled within the southeastern U.S. looking for populations of *I. glabra* and *I. coriacea*. Leaves containing well-developed mines were removed from plants, and adult flies were reared from the pupae found within the mines. Species identity of the leafminers reared from *I. glabra* and *I. coriacea* was determined using Kulp (1968) and Spencer and Steyskal (1986).

RESULTS

Adult flies reared from *I. glabra* and *I. coriacea* were readily identified as *P. glabricola*. Flies reared from the two hosts did not noticeably differ from each other in morphological characters. Locations where *P. glabricola* was reared from leafmines on *I. glabra* and/or *I. coriacea* are listed in Table 1. Collection dates for samples are listed in Table 1; eclosion of adults occurred within approximately one month of this date.

Leafmines formed by *P. glabricola* on both *I. glabra* and *I. coriacea* are linear-blotch shaped, although in most cases the extensive feeding during the later instars removes tissue surrounding the initial linear mine so that only a blotch is apparent. Generally, a leaf will contain only one mine

with a single larva or pupa present. Occasionally, a leaf will have more than one mine, indicating the presence of several larvae or pupae. This appeared to be more common on the larger-leaved *I. coriacea* than on *I. glabra*. Mines on *I. glabra* have a tendency to be brown with a blistered appearance, while those on *I. coriacea* are more often dark green or reddish-green and do not appear blistered. On both hosts, fly pupation occurs in the mine with the anterior pupal spiracles emerging through an "epidermal window," where just prior to pupation, the larva removes mesophyll adjacent to the lower epidermis of the leaf.

During the course of this study, it became apparent that populations of *P. glabricola* on *I. coriacea* exhibit a univoltine life cycle. Well-developed mines are only found in January and February, and the adults eclose from late January to early March. Observations made at other times indicate that first-instar leafminers are found in the summer, second-instar mines in late fall, and third-instar mines only in the winter prior to adult eclosion. This univoltine life cycle is very similar to that of the native holly leafminer *P. ilicicola* on its host *I. opaca* (Kulp 1968, Potter and Kimmerer 1986), but it is in sharp contrast to the multivoltine life cycle of *P. glabricola* on *I. glabra* (Kulp 1968, Al-Siyabi and Shetlar 1998), where the generation time can be as short as one month (Scheffer, personal observation).

DISCUSSION

These findings confirm that *I. coriacea* is a widely used host for *P. glabricola* in natural settings and extends the known geographic distribution of *P. glabricola* to Alabama, Florida, Mississippi, North Carolina, and South Carolina.

It is unlikely that further study will uncover any additional hosts for *P. glabricola*. Most holly leafminers are strictly monophagous (Kulp 1968, Scheffer and Wiegmann 2000), although in artificial situations (e.g., botanical gardens), additional feeding on

non-native *Ilex* species or hybrids may be observed (Scheffer, personal observation; host records listed in Spencer and Steyskal 1986, Griffiths and Piercey-Normore 1995). During the course of this study, *P. glabricola* was reared from *I. glabra* and *I. coriacea* in both natural and ornamental settings, but not from other hollies growing in the same locations, including the evergreen species *I. cassine* Linnaeus, *I. myrtifolia* Walter, *I. opaca* Aiton, and *I. vomitoria* Aiton (Scheffer, unpublished data).

Although holly leafmining species are known to exhibit different patterns of voltinism, no species previously has been reported to exhibit more than one pattern. Many holly leafminers have a multivoltine life cycle with two or more generations a year. In most of these species, the larval feeding period may be as short as a week or two, with a total generation time that of slightly over a month (Scheffer, personal observation; the life cycle of *P. opacae* Kulp is reported to be even shorter (Kulp 1968)). Multivoltine holly leafminers can be found on either evergreen or deciduous hollies and include the species *P. ditmani* Kulp, *P. opacae* Kulp, *P. verticillatae* Kulp, *P. vomitoriae* Kulp, and several undescribed species (Kulp 1968, Spencer and Steyskal 1986, Scheffer, unpublished data). In contrast, univoltine holly leafminers have only one generation a year and a highly extended larval developmental period lasting up to ten months that appears to be unique among the leafmining Agromyzidae (Cameron 1939, Hering 1951, Potter and Kimmerer 1986). Univoltine holly leafminers have been found only on evergreen hollies and include the species *P. ilicicola* Loew, *P. ilicis* Curtis, and an undescribed species on *I. myrtifolia* (Kulp 1968, Spencer and Steyskal 1986, Scheffer, unpublished data).

There are several possible explanations for the observation that *P. glabricola* has a different life history pattern depending on host plant identity. At the two extremes, these hypotheses can be classified as those involving phenotypic plasticity and those

involving genetic differences between flies on the two hosts. First, the rate of larval feeding could be a direct physiological result of some unknown quality of host leaf tissue, such that *P. glabricola* larvae in *I. glabra* always develop quickly, while larvae in *I. coriacea* develop very slowly. This situation would be a form of host-mediated phenotypic plasticity and would not require genetic differences in the flies. Consideration of the life history patterns of related holly leafminers suggests that an hypothesis of host-mediated phenotypic plasticity, even if found to be valid in the case of *P. glabricola*, cannot generally explain voltinism differences in the holly leafmining group; *P. illicicola* and *P. opacae* both oviposit into the soft new leaves of *I. opaca*, but *P. illicicola* exhibits a univoltine life cycle on this host while *P. opacae* is multivoltine (Kulp 1968, Scheffer and Wiegmann 2000).

An alternative hypothesis is that the flies exhibit different patterns of voltinism because they are genetically different and represent host races or incipient or cryptic species. Under this scenario, the difference in life history on the two hosts could be either a cause of genetic divergence (i.e., the life history differences may be incompatible adaptations to each of the two host plants thereby favoring host race formation) or a result of genetic divergence (i.e., host races diverged for reasons unrelated to voltinism patterns and voltinism patterns have subsequently diverged). Note that even if genetic host races have formed, the difference in voltinism could still be due to phenotypic plasticity, if it exists, such that voltinism would depend on the host, but each host race would exhibit only one pattern because it uses only one host.

Whether *P. glabricola* on *I. glabra* and *I. coriacea* represents a single oligophagous species that feeds on two hosts or a complex of closely related but genetically divergent host races currently is not known. Although the difference in voltinism on the two hosts is suggestive of host races, the

voltinism difference by itself does not preclude flies from the two hosts from interbreeding. Adult flies from both hosts emerge in late winter (see dates in Table 1) and can be expected to be present in the same general areas together for several weeks. During this one time each year, interbreeding between flies from the two hosts is at least possible.

Anecdotal evidence from the field indicates that host plant preferences suggestive of host races may be present within *P. glabricola*. When the univoltine leafminers on *I. coriacea*, which are often extremely abundant, emerge en masse in February, adults can be readily observed on *I. coriacea* foliage but not on adjacent *I. glabra* foliage (Scheffer, personal observation), suggesting a possible preference for *I. coriacea*. This is despite the fact that *P. glabricola* from *I. glabra* can be taken into the lab and easily reared to the next generation on *I. glabra* with no apparent hesitancy to oviposit and little or no larval mortality (Scheffer, personal observation).

Results to date of phylogenetic analysis of molecular data are inconclusive with regard to the question of host races within *P. glabricola*. Mitochondrial cytochrome oxidase sequences of flies from *I. glabra* and *I. coriacea* are nearly identical (< 1% pairwise divergence across more than 2000 bp; Scheffer and Wiegmann 2000), which is typical of sequence variation found within agromyzid species (Scheffer, unpublished data; see also Scheffer 2000). Using this gene region, there is no evidence of host-associated divergence, but given the estimated rate of evolution of insect mitochondrial genes (approx. 2.3% per million years; Brower 1994), mitochondrial sequences would be unlikely to have sufficient variation to track very recent divergence or speciation events.

In conclusion, *P. glabricola* is widely distributed throughout the coastal plain of the eastern United States. It feeds on two holly species, *I. glabra* and *I. coriacea*, which are commonly sympatric throughout

much of their ranges. Differences exhibited by *P. glabricola* in patterns of voltinism on these two hosts suggest either host-associated phenotypic plasticity in life history or the presence of genetically differentiated host races. Additional studies of morphological variation, mating and oviposition behaviors, and/or highly variable molecular markers are needed to determine the status of *P. glabricola* leafminers feeding on *I. glabra* and *I. coriacea*.

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